UNIVERSITI TEKNOLOGI MARA

PROBIOTIC FERMENTED SOYMILK AS A DIETARY ANTIOXIDANT SOURCE AND ANTIPROLIFERATIVE AGENT

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AUTHOR'S DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Teknologi MARA. It is original and is the result of my own work, unless otherwise indicated or acknowledged as referenced work. This thesis has not been submitted to any other academic institution or non-academic institution for any other degree or qualification.

I, hereby, acknowledge that I have been supplied with the Academic Rules and Regulations for Post Graduate, Universiti Teknologi MARA, regulating the conduct of my study and research.

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ABSTRACT

There is accumulating evidence ascribing the health potential of biologically active aglycone isoflavones in soy. The isoflavones in soy however exist predominantly in the inactive glucoside form. Several studies strongly supported the action of probiotics, mainly lactic acid bacteria (LAB) that possess β-glucosidase which hydrolyses glucosides to biologically potent aglycones during soymilk fermentation. In the present study, 12 LAB (lactobacilli and pediococci) isolated from Malaysian fermented food or milk products that possess probiotic characteristics were evaluated for their ability to ferment soymilk and hydrolyse glucosides to biologically potent aglycones. The soymilk fermented with LAB was assessed for their antioxidant properties *in vitro* and *in vivo*. Soymilk fermented with selected LAB were further assessed for antiproliferative effect *in vitro* and inhibition of aberrant crypt foci (ACF) formation in azoxymethane (AOM)-induced colonic neoplastic rats. The safety status of soymilk fermented with LAB was determined through acute and subchronic toxicity studies. The unfermented (negative control) and soymilk fermented with *L. casei* LABPC (positive control) were used as controls in the present study. The results showed that soymilk supported the growth of LAB. The level of bioactive aglycones in soymilk was enhanced ($P<0.05$) between 50 to 90% after 48 h of fermentation with the 12 LAB or LABPC. The total phenol content increased ($P<0.05$) only in soymilk fermented with *L. fermentum* LAB 9 (58.68 mg gallic acid/100 mL) as compared to the unfermented soymilk (51.92 mg gallic acid/100 mL). DPPH radical-scavenging activity of soymilk fermented with the 12 LAB (75-100%) was significantly ($P<0.05$) greater than that of the unfermented soymilk (62%) at 48 h. The ferrous ion chelating effect was enhanced ($P<0.05$) in soymilk fermented with *Lactobacillus* sp. LAB 1 (65%), LAB 12 (63%), *P. pentosaceus* LAB 7 and LAB 8 (64-67%) after 48 h of incubation. The ethyl acetate extracts of supernatant (EAS) and cell fractions (EAC) of LAB fermented soymilk exhibited significantly ($P<0.05$) higher antiproliferative effect as compared to the unfermented soymilk extracts against human colorectal cancer cells (HCT116 and HT-29). The soymilk fermented with *P. pentosaceus* LAB 8 and *Lactobacillus* sp. LAB 12 that exerted strong antiproliferative effect *in vitro* also showed chemopreventive effect against colon carcinogenesis in AOM-induced rats. Total number of ACF was significantly ($P<0.05$) lower in rats fed with soymilk fermented with *P. pentosaceus* LAB 8 and *Lactobacillus* sp. LAB 12 (77-87%) than that of the control (saline, 57-66%) or LABPC group (73-74%). *In vivo* antioxidant activities (SOD, CAT and GPx) were enhanced in AOM-induced rats fed soymilk fermented with LAB or LABPC. The LAB fermented soymilk also showed hypocholesterolemic effect. Furthermore, incorporation of LAB in the diets at the dose of 10^9 and 10^{10} cells daily for 28 days did not show any significant differences on behavior, general health status, body weight and blood analysis in all the treated groups as compared with control, suggesting that the safety characteristics of the LAB strains (LAB8 and LAB 12). This study indicates that LAB has the potential to enrich bioactive isoflavones in soymilk and possess promising antioxidative and antiproliferative activities to improve the nutritional value of soymilk.

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